A Study of *Sclerospora Graminicola* (Sacc.) Schroet. on *Setaria Viridis* (L.) Beauv. and *Zea Mays* L.

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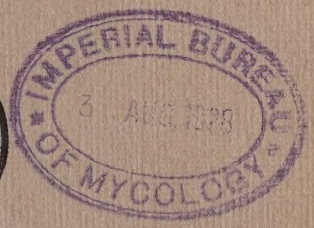

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SUMMARY

Conidial sporulation of *Sclerospora graminicola* has been observed in the day time occurring naturally in the field and under artificial conditions in the laboratory. The period required for the development of mature conidia lies between 4 hours 35 minutes and 11½ hours. The conditions which seem necessary for the production of conidia, whether during the day or night, are: a completely saturated atmosphere, turgid host leaves, a slight moisture film on the surface of the leaves and a temperature ranging between 8° and 27°C.

When flooded into a drop of water immediately after being discharged, conidia were found to germinate after 60 minutes. The best germination was obtained at 15°C., but the optimum was not definitely determined.

Normal conidia measure 14-23 x 11-17μ. Sometimes larger conidia are produced (43 x 18.6μ).

The average length of conidiophores of *Sclerospora graminicola* was found to be 267.8μ while the individuals measured ranged from 214.5 to 375.3μ, a variation of 160.8μ.

Spores were found to be forcefully discharged from the conidiophores thru a distance of 2.5 mm. vertically and 1.89 mm. horizontally. *Setaria viridis, S. italica, Zea mays* (May's Golden popcorn) and *Euchlaena mexicana* were infected with *Sclerospora graminicola* when exposed to the conidia of the mildew.

Oospores of *Sclerospora graminicola* were found to overwinter naturally in field soil under Iowa conditions. In one test, oospores which overwintered outdoors gave nearly twice as much infection on *Setaria viridis* and *Zea Mays* (Japanese Hulless popcorn) as did the oospores which were kept in the laboratory.

Plants from five genera of Gramineae were found to be susceptible hosts to *Sclerospora graminicola*. These are: Euchlaena, Setaria, Holcus, Saccharum and Zea. *Setaria viridis* was found to be the most susceptible of all hosts and popcorn more susceptible than sweet corn and dent corn.

Six days were found to be the usual period of incubation between the time oospores were placed on the seeds and that when conidial fruiting appeared on the leaves.

Infection by oospores was obtained from the time the testa was broken until the emergence of the plumule above ground. Relative susceptibility of seedlings decreases with age. The processes connected with infection are more greatly favored by temperatures of 15° to 16°C. than by temperatures of 24° to 30°C.

The germinating oospore is evidently unable to penetrate older leaf tissue.

The viability of oospores was little affected by soaking in 2 percent copper sulfate solutions for 10 minutes, while similar treatment in 1 percent formaldehyde for 5 minutes proved fatal. The killing action of mercuric chloride 1-1000 was not so great as that of formaldehyde.

Freshly collected oospores which were held in a dry condition at 77°C. for 1 hour later gave 52 percent infection on *Setaria viridis*, while wet spores lost their viability to a marked degree when held at 50°C. for a similar period.

Sclerospora graminicola was studied in the field during the summers of 1925, 1926 and 1927. Infection was obtained on corn and teosinte planted in plots which had been artificially infested with oospores. Spontaneous conidial sporulation was found to be comparatively rare on corn in the field altho it was observed in 1926 and 1927 on young seedlings during periods of high humidity and cool temperatures. Infected plants were either killed outright or became stunted and unproductive. A few plants apparently outgrew the attack.

In Iowa, *Sclerospora graminicola* has been observed only twice occurring naturally on corn in the field.

Oospores of *Sclerospora graminicola* which had been held 30 months under dry conditions in the laboratory were found to be viable.

Presoaking of oospores does not seem to affect the percentage of infection.

Soil is not necessary as a medium for the germination of oospores.

NOTE: Much confusion exists in the use of several scientific names found in this paper. The following synonomy gives the nomenclature found in common usage:

Setaria viridis (L.) Beauv. = *Chaetochloa viridis* (L.) Scribn.
Setaria glauca (L.) Beauv. = *Chaetochloa glauca* (L.) Scribn. and *Chaetochloa lutescens* (Weigel) Stuntz.
Setaria magna (Griseb.) = *Chaetochloa magna* (Griseb.) Scribn.
Setaria verticillata (L.) Beauv. = *Chaetochloa verticillata* (L.) Scribn.
Setaria italica (L.) Beauv. = *Chaetochloa italica* (L.) Scribn.
Holcus sorghum L. = *Andropogon sorghum* Brot. and *Sorghum vulgare* Pers.
Pennisetum typhoideum Rich. = *Pennisetum glaucum* (L.) R. Br.

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The genus Sclerospora includes many species that attack the cereals. Altho these downy mildews are more commonly found in the Orient than in America, *Sclerospora graminicola* (Sacc.) Schroet. is generally known in this country on *Setaria viridis* (L.) Beauv., a weed common in corn fields. This fact led to studies to determine if *Zea mays* L. and other cereals are susceptible. The ease with which oospore infection took place in the seedling stage on *Setaria viridis* and *Zea mays* stimulated further study of the conidial and oospore response to environmental conditions. An attempt was made also to determine the conditions favorable to the growth and development of the pathogene including studies of infection and host range.

LITERATURE REVIEW

Sclerospora graminicola (Sacc.) Schroet. on *Setaria viridis* (L.) Beauv. was described first by Farlow (6) in 1884. He noted the precise similarity between the oospores on specimens collected in Wisconsin and those collected in Europe and described by Schroeter (16).

The malformation of the heads of *Setaria viridis* due to Sclerospora was described by Halsted (7, 8), who observed the direct relation between rainfall and prevalence of downy mildew in 1886 and 1888. Both Hungarian grass *(S. italica* (L.) Beauv.) and green foxtail *(S. viridis)* were found to be hosts in Iowa.

Trelease (20) illustrates the shredding of the leaves of *Setaria italica* and *S. viridis* due to oospore formation within the tissues. He comments that until 1896 the disease had not been seriously destructive.

Pammel, Weems and Lamson-Scribner (12) report *Sclerospora graminicola* to be commonly distributed thru Iowa and Nebraska. They also possessed specimens collected in North Dakota by Prof. H. L. Bolley.

Wilson (26) reports *Sclerospora graminicola* on *Setaria italica and S. viridis* ranging from Vermont to South Dakota and Kansas. In a later paper (27) he adds *S. glauca* (L.) Beauv. to the list of host plants.

In his list of parasitic fungi in Wisconsin, Davis (4) reports *Sclerospora graminicola* on *Setaria viridis* and *S. italica.*

In America, new impetus was given to the economic study of this downy mildew when Melhus and Van Haltern (10) announced the results of their preliminary work on *Sclerospora graminicola* of corn *(Zea mays* L.). This is the first work done in the United States to identify *Sclerospora graminicola* with corn, altho six species of the genus Sclerospora had been previously found causing diseases of corn in Europe, South America and the Orient. In 1909 Spegazzini (19) reported the fungus in the male panicles of *Zea mays* in marshes in Isle Santiago near La Plata, Argentina.

From Java, Raciborski (14) describes a destructive disease of corn caused by *Peronospora maydis* Rac. It is called "lijer" or "sleepy" disease by the natives. In some localities it reaches epidemic intensity. Young plants become diseased and in some cases fall over and die. The first two or three leaves are wholly green and without infection, but the fourth and later leaves show white, whitish-yellow, or whitish-green markings. Infection takes place either thru entire leaves or in bands. The leaves are packed with non-septate mycelium. Conidial infection of young corn is produced in 8 to 12 days as evidenced by great numbers of conidiophores protruding from the stomata. Spherical oospore-like bodies are commonly found in the leaf sheaths, but their germination has not been observed. Raciborski comments upon the uncertain origin of the disease, inasmuch as corn is believed not to be a native of the Orient, but to have been carried to Java by the Portugese in 1496. Inspection of the native grasses did not reveal the mildew.

Shirai (17) gives a detailed account of *Sclerospora graminicola,* the most common and most destructive disease of *Setaria italica* found in Japan. In severe cases crops are reduced to less than 50 percent. Diseased plants exhibit the peculiarity of bearing oospores and conidia separately on different leaves and organs. Oospores are found either in the metamorphosed floral leaves of the deformed heads or in the etiolated leaves on the upper part of the stem, the uppermost of which are usually rolled and remain unexpanded. Conidia are borne on the under surface of the green leaves which grow on the lower part of the stem. In diseased leaves the walls of the mesophyll cells are less thickened and less silicious than those in normal tissues, thus giving rise to shredded leaves, which is one of the characteristic symptoms of the disease. Oospores have large central oil drops, granular protoplasm and eccentrically placed refractory bodies of unknown nature. A medium-sized leaf is estimated to contain 3,500,000 oogonia. Measurements of fruiting structures agree with those of Schroeter (16), except that a second type of conidium is produced. Conidia of a roundish-

oval shape measure 24-28.8 by 16.8-19.2 μ. These agree with Schroeter's description. Spores of the second type are oblong and measure 38.4-57.6 by 19.2-24 μ.

An excellent discussion of the pathological effects of *Sclerospora graminicola* on *Pennisetum typhoideum* Rich. is given by Butler (2). This mildew is one of the few plant parasites which cause decided alterations in the reproductive parts. The elements of the inflorescence undergo unusual hypertrophies which often result in sterility. Conidia are produced only on the normal leaves, while the oogonia develop both in the tissues of the leaves and heads. The conidiophores measure 100 by 12-15μ and the conidia are 22-30 by 12-16μ. Following germination, the conidia give rise to zoospores, the number ranging from 3 to 12 according to the size of the conidium. The zoospores are irregularly kidney-shaped, unequal sided, flattened bodies with two cilia arising from the concave side. After a period of activity, the zoospores become quiescent, round off into spheres 9 to 12μ in diameter and rapidly germinate by hyphae. The oogonia are formed in great numbers within the mesophyll tissue. Often they are arranged in longitudinal rows which lie on both sides of the leaf veins. The number of oogonia formed beneath one square millimeter of leaf surface is estimated to be no less than 480. Measurements made on the oogonia give an average diameter of 42μ, while the oospores within average 32μ in diameter. The oospores have not been observed to germinate, altho from the evanescent nature of the asexual stage and the regular manner in which the disease appears, it is highly probable that germination occurs freely in nature under suitable conditions.

Observation on three other diseases caused by Sclerospora are reported by Butler. He records these hosts of Sclerospora as *Pennisetum typhoideum, Andropogon sorghum* and *Euchlaena luxurians,* but believes the causal agent to be the same as *Sclerospora graminicola.*

Butler (3) reports the appearance of *Peronospora maydis* Rac. in India in 1912. He states that the lower leaves are usually normal, but the upper part of the plant is chlorotic, owing to the absence of chlorophyll in long streaks. Plant growth is checked and the internodes are frequently shortened so as to give a bunchy appearance to the head. Little if any grain is produced, altho the tassel may be quite normal. Infected plants wither and die some weeks before the normal ones. No oospores have been found. The spherical bodies reported by Raciborski have been determined to be the resting stage of a protozoan. Butler designates the fungus found in India as *Sclerospora maydis* (Rac.) Butl.

In "Additional Notes on Philippine Plant Diseases" by Baker (1), the following paragraph is given: "The most im-

portant feature in connection with corn culture in the Philippines during the past few years has been the recognition of the presence and enormously destructive power of the Indian corn mildew, due, we suppose, to *Sclerospora maydis* (Rac.) Butl. It attacks the young plants, which become chlorotic and fail to develop. Ten percent and above is commonly lost thru this disease, while in some cases whole fields may be swept clean. In one interesting case, a part of a field had been subjected to wash during a heavy storm just after planting. On this area every plant was taken by the mildew, while in the remainder of the field not a half dozen died.''

In a later publication, Reinking (15) further describes the downy mildew of maize in the Philippines. He considers the causal fungus there to be identical with that described by Butler in India but different from *Sclerospora javanica* (Rac.) Palm. Young plants, from 10 to 60 centimeters in height, show first evidences of disease in the upper leaves, which have white stripes running parallel to the margins and frequently are entirely whitened. Masses of conidiophores on both the upper and lower surfaces of the leaves cause the infected plants to appear pale at a distance. Such plants are stunted and have a bunchy growth, due to the checking of growth and frequently to the shortening of the internodes. Often no grain is produced, and the male inflorescence is produced prematurely. Badly diseased plants wither and die before the normal plants have matured. In severely infected fields, 60 to 70 percent of the crop may be destroyed.

Palm (11) defines three distinct manifestations of the ''lyer'' (lijer) disease of corn in Java caused by *Sclerospora javanica* (Rac.) Palm. (1) Plants attacked by the mildew remain small. The leaves are narrow and are of a yellow or greenish yellow color and, because of their poorly developed root systems, such plants often lie down. (2) The plants are of normal development but with yellow striping on the leaves. The stripes arise from a common base in the lower part of the leaf. (3) Diseased plants are of normal appearance except for narrow, sharply defined stripes of yellow or brown on the basal leaves. The stripes very seldom anastomose at their basal part.

The fungus is not identical with the Sclerospora described by Butler. The conidium germinates with one or more germ tubes which penetrate the leaf thru the stomata. Spores lying exposed on the leaf are viable for one day but when on or in the ground, viability is held over a longer period, tho not exceeding four days. Wind is the most important agent of dissemination. Oospores have not been found, and the mycelium, which is sometimes present in seed, does not give rise to the disease. Incubation after conidial inoculation extends from 10 to 20 days, but one month is not uncommon. Susceptibility increases until

three or four leaves are unrolled, then decreases rapidly so that plants three weeks old are apparently not susceptible.

Weston (21, 22) has since described two species of Sclerospora in the Philippines. *Sclerospora philippinensis* Weston is highly infectious to corn. He describes it as follows: ". . . . the disease may be said to manifest itself by the loss of chlorophyll in more or less sharply defined areas of the leaf, but the production of a whitish down of conidiophores principally on the chlorotic area, and by a more or less extensive alteration in the form or the normal growth of the plant. The change in color is the most striking and obvious symptom. Since, however, somewhat similar changes in color and form may result from other causes, the characteristic downiness is the surest indication of the disease."

Weston further describes the pale, yellowish streaking of the leaves and their characteristic stiffening. The plants are dwarfed by the checking of growth in the internodes and, as the growth of the leaf sheaths is not decreased proportionally to that of the stalk, the sheaths commonly overlap. A wide range of malformations has been observed in fruiting structures. Sterility is one of the most serious effects. Susceptibility decreases markedly with age. Plants harboring the mycelium of the parasite may never form conidiophores unless environmental conditions are favorable. Some plants may regain their normal color late in the season while others will be killed early or struggle along until a few weeks before harvest time. No oospores are known in connection with *Sclerospora philippinensis.*

The second mildew described in the Philippines, *Sclerospora spontanea* Weston, is reported to produce symptoms on corn which in general appearance are identical with those of *S. philippinensis.* This species occurs on the wild grass, *Saccharum spontaneum* L., on sugar cane and corn.

Oogonial stages of Sclerospora were encountered on *Saccharum spontaneum* L., *S. officinarum* L., (sugar cane) and *Miscanthus japonicus* (Thunb.) Anders. The relation of these oospores to the conidial stages of the Philippine mildew has not been determined.

The disease caused by *Sclerospora graminicola* on corn in America has much in common with the downy mildew diseases of corn in the Orient. Melhus and Van Haltern report a grayish blotching and mottling of the leaves which may extend thruout the whole plant. In other cases, only a few mottled yellow spots develop, or chlorotic areas occur in the form of longitudinal stripes. Symptoms usually appear within 10 days after the plumule emerges. Stunting always occurs, the amount depending on the severity of the infection. Some plants die when only 3 inches tall, while a few appear to outgrow the disease. Many

TABLE I. DISTRIBUTION AND MORPHOLOGICAL CHARACTERS OF THE DESCRIBED SPECIES OF SCLEROSPORA

Sclerospora spp.	Host range	Reported	Conidiophores		Spores	Conidial germination	Oospores
			Length	Basal cell			
S. graminicola (Sacc.) Schroet.	*Setaria viridis* (L.) Beauv.	U.S., Europe	267.8μ	Absent	Abundant	Zoospores	Abundant;
	Setaria italica (L.) Beauv.	U.S., Japan, India			14-23x		30-60μ
	Setaria magna Griseb.	U.S.			11-17μ		*a*
	Setaria glauca (L.) Beauv.	U.S., Europe					
	Setaria verticillata (L.) Beauv.	Europe, U.S., Asia					
	Zea mays L.	U.S., Argentina					
	Panicum miliaceum L.	U.S.					
	Euchlaena mexicana Schrad.	U.S., India					
	Holcus sorghum L.	India, U.S.					
	Saccharum officinarum L.	U.S.					
	Pennisetum typhoideum Rich.	India					
S. magnusiana Sor.	*Equisetum* sp.	Russia	*		Unknown	Unknown	45-50μ
S. miscanthi T. Miy.	*Miscanthus* sp.	Japan	*		Unknown	Unknown	Known
S. farlowii Griff.	*Chloris elegans* H.B.K.	Arizona, Sonora	*		Unknown	Unknown	28-45μ
S. macrospora Sacc.	*Alopecurus* sp.	Australia	*		Unknown	Unknown	60-65μ
	Triticum aestivum L.	U.S., Europe					
	Zea mays L. *b*	Europe					
	Bromus commutatus Schrad.	U.S.					
S. javanica (Rec.) Palm.	*Zea mays* L.	Java	Average	Present	19-26x	Germ tube	Unknown
	Corn-teosinte hybrids	Java	300μ		15-20μ		
S. maydis Butl.	*Zea mays* (L.)	India, Philippine	150μ		28-45x	Germ tube	Unknown
	Euchlaena sp.	Islands			16-22μ		
S. sacchari T. Miy.	*Zea mays* L.	Formosa			25-41x	Germ tube	Reported, but
	Euchlaena sp.	Formosa			15-23μ		connection
	Saccharum officinarum L.	Formosa, Queensland, Fiji Islands, Philippine Islands					uncertain
S. philippinensis Weston	*Zea mays* L.	Philippine Islands	150-400μ	Present	27-39x	Germ tube	Unknown
	Euchlaena mexicana Schrad.	Philippine Islands			17-21μ		
	Holcus sorghum L.	Philippine Islands					
S. spontanea Weston	*Zea mays* L.	Philippine Islands	350-550μ	Present	39-45x	Germ tube	*c*
	Saccharum spontaneum L.	Philippine Islands			15-17μ		
	Saccharum officinarum L.	Philippine Islands					
	Sorghum	Philippine Islands					
S. graminicola var. *andropogonis sorghi* Kulk	*Holcus sorghum* L. (jowar) *f*	British India	*d*		18-32x 16-23μ	Germ tube	*e*

*Conidiophores have not been observed.

*a*Average diameter of oogonium 42μ.

*b*S. *macrospora* has also been reported on *Phalaris arundinacea*, *Ph. coerulescens* (?), *Ph. canariensis* L., *Agropyrum repens* (L.) Beauv., *Glyceria maritima* (?), *Lolium perenne* L., *Agrostis palustris* Huds. (?), *Holcus mollis* (?), *Phragmites communis* Trin., *Avena fatua* L., oats, barley and rice in Europe.

*c*Connection with oospores found on *Saccharum spontaneum* L. uncertain.

*d*Maximum length of papillae 16.3μ.

*e*Average diameter of oogonium 41μ.

*f*S. *graminicola* var *andropogonis sorghi* has also been reported on *Pennisetum typhoideum* Rich (bajri), *Setaria italica* (L.) Beauv. and *Euchlaena mexicana* Schrad. in India.

plants become intensely green instead of developing chlorotic areas after the initial symptoms appear. A bushy, stocky appearance is characteristic, due to the production of the normal number of leaves on a stalk with shortened internodes. Conidial sporulation is rather sparse, while oospore production has never been found on corn.

Weston and Weber (25) have recently identified *Sclerospora graminicola* on *Chaetochloa magna* (Griseb.) Scribn. in the Everglades of southeastern Florida. All measurements indicate that the fungus is similar to that found in the northern states. Systemic infection is usual, but localized spots of infested tissue are sometimes found. The shredding of leaves and of inflorescence is accompanied by the formation of great numbers of oogonia.

DISTRIBUTION AND MORPHOLOGICAL CHARACTERS OF THE DESCRIBED SPECIES OF SCLEROSPORA

In the genus Sclerospora 10 species and 1 variety have been described. Seven of the ten species are reported as attacking corn, two sorghum and six teosinte. Altho species of the genus are widely distributed geographically, the genus has more representatives in the Orient than elsewhere (table I).

It is interesting to note that the type species of the genus, *Sclerospora graminicola,* is the only one that has been found to germinate by zoospores. The variety of Sclerospora described by Kulkarni as *S. graminicola* var. *andropogonis sorghi* is strikingly different in this respect. The fact that oospores and conidia are not produced on all the different hosts listed suggests that certain differences between species may be eliminated with further study of the genus thru cross-inoculation.

STUDIES OF THE CONIDIAL AND OOGONIAL SPORE FORMS OF SCLEROSPORA GRAMINICOLA

The following pages present studies on the hosts relationships of the conidial and oogonial stages of *Sclerospora graminicola.* The detailed data of conidial response will be considered first.

CONIDIAL RESPONSE

Further information was obtained from the studies of the asexual stage of Sclerospora which included the influence of environment upon the production of conidia, the time required for sporulation and germination of conidia, the role of tempera-

ture in conidial germination and measurements of the asexual fruiting organs.

DAYTIME PRODUCTION OF CONIDIA

Data from 14 tests made upon the conidial sporulation of *Sclerospora graminicola* are given in table II. Diseased leaves of *Setaria viridis* were collected in the field and immediately taken into the laboratory. They were wiped free from conidiophores by drawing them thru folded cheese cloth until no trace of the original sporulation remained. The leaves were moistened again by drawing them thru wet fingers or cloth. They were then placed in moist chambers.

Sporulation developed during periods ranging from 4 hours and 35 minutes to 11½ hours. In all cases daylight was present thruout the formation of the conidia.

Under field conditions the daytime production of conidia also has been observed. The forenoon of August 6, 1925, was cloudy but not damp. The grass leaves were quite dry. A gentle rain started about 1:30 p.m. and continued during the afternoon. This changed into a heavy downpour from 6:00 p.m. to midnight. At 6:30 p.m. leaves of *Setaria viridis,* white with conidiophores, were collected in the field. Microscopic examination revealed great numbers of mature conidia. Water cultures of the conidia were made and by 6:30 a.m. the following morning 70 percent of the conidia had germinated.

In this case the survival of conidia thru the dry hours of the morning was unexpected since they are easily killed by drying. Furthermore, the conidia had just become mature at the time of collection as was shown by the lack of empty conidia and

TABLE II. OBSERVATIONS ON THE PRODUCTION OF CONIDIA IN THE DAYTIME

Date	Temp. damp chamber (deg.)	Time started	Time viable conidia appeared	Interval (hours)	Conidial germination (percent)
1924					
Aug. 19	18	11:40 a.m.	7:40 p.m.	8	....
Aug. 24	18	10:20 a.m.	6:30 p.m.	8 1/6	....
Aug. 25	18	8:30 a.m.	4:45 p.m.	8 1/4	....
Aug. 26	18	12:30 p.m.	7:30 p.m.	7	....
Aug. 26	18	10:10 a.m.	5:30 p.m.	7 1/3	....
Aug. 28	18	8:45 a.m.	5:00 p.m.	8 1/4	....
Sept. 5	18	9:45 a.m.	7:30 p.m.	9 3/4	....
Sept. 6	18	8:30 a.m.	6:30 p.m.	10	....
Oct. 14	18	10:00 a.m.	6:00 p.m.	8	....
Oct. 14	18	9:50 a.m.	6:20 p.m.	8 1/2	60
1925					
March 16	18	8:00 a.m.	12:35 p.m.	4 7/12	....
March 20	18	8:00 a.m.	5:05 p.m.	9 1/12	....
April 27	....	8:00 a.m.	7:30 p.m.	11 1/2	....
Aug. 6	....	.....	.....	5	70

the presence of only three or four zoospores in all of the material studied. If the conidia had matured earlier there would have been an abundance of empty conidia and many zoospores. In this instance conidia were produced in 5 hours during the daytime and in the open.

The foregoing studies indicate that neither the absence of light nor the time of day are limiting factors in conidial production. On the contrary, the fungus will sporulate at any time after reaching the fruiting stage (6 to 10 days from inoculation) and before the formation of oospores if given the following environmental conditions: a completely saturated atmosphere, turgid host leaves, a slight moisture film on the surface of the leaves and a temperature ranging approximately between 8° or 10° to 27°C. Too much as well as too little moisture on the leaves will inhibit sporulation. These findings do not seem to be in accord with Weston (24) who found that this fungus produces conidia only during the night.

TIME REQUIRED FOR THE DEVELOPMENT OF CONIDIA

The behavior of *Sclerospora graminicola* under favorable environmental conditions was studied further, and the results are recorded in table III. In all trials except the first, infected leaves of *Setaria viridis* were wiped clean of conidiophores with

TABLE III. THE TIME REQUIRED FOR THE DEVELOPMENT OF CONIDIA OF *SCLEROSPORA GRAMINICOLA*

Date	Treatment of leaves	Temperature (deg. C.)	Time in hours: Mildew detected	Time in hours: Conidia mature	Time in hours: Conidia discharged	Percent germination
1924						
Aug. 19	Placed in damp chamber	24–26	.....	8	.....	....
Aug. 24	Wiped and placed in damp chamber	26	.....	8	.....	60
Aug. 25	Wiped and placed in chamber under running water	18	.....	8	.....	50
Aug. 26	" "	18	6¾	7	.....	76
Aug. 26	" "	20	6	7½	.....	71
Aug. 28	" "	18	7	8	.....	75
Sept. 5	" "	18	.....	9¾	.....	95
Sept. 6	" "	18	7	8¾	10	....
Sept. 6	Wiped and placed in double slide under tap	18	7	8¾	10	
Oct. 14	" "	18	.....	.....	8	....
Oct. 14	Wiped and placed in damp chamber under running water	18	8	8¾	.....	60
1925						
Mar. 16	Wiped and placed in double glass slides in moist chamber	.....	4½	.....	4 2/3	....
Mar. 20	" "	18	7½	.....	9 1/12	....

a cloth. In order to obtain a film of water over the leaves, it was found necessary to place the moist chambers containing them under cold running tap water. The initial warmth of the chambers aided in bringing about the deposition of the moisture film. Three tests were made in which the leaves were placed between double glass slides. These covers were about 3 millimeters apart. This arrangement was such that the conidia were caught on the under surface of the upper slide as they were discharged.

It seems probable that moisture is the most important factor influencing sporulation. In the tests where 9 or more hours were required, the surfaces of the leaves may not have been sufficiently moist for several hours after the beginning of the test. Sporulation already had been shown to occur in nature within 4 to 6 hours after suitable moisture conditions prevail. Weston (23) finds that *Sclerospora spontanea* and *S. philippinensis* sporulate within 5 to 7 hours.

TIME REQUIRED FOR THE GERMINATION OF CONIDIA

Fresh conidia were secured by suspending a moist cover glass above a sporulating leaf. As the conidia were discharged from the conidiophores, they were caught on the glass. The spores were immediately flooded into a drop of water and observed under the microscope.

Faint protoplasmic lines were observed after 40 minutes. Development within the spores then progressed rapidly, for at 45 minutes the lines were distinct and at 60 minutes the zoospores were swimming. This test was conducted at a room temperature of 20° to 22°C.

INFLUENCE OF TEMPERATURE ON GERMINATION OF CONIDIA

Quantities of fresh viable conidia were obtained by placing glass slides under actively sporulating leaves. These were washed into a small amount of distilled water to make up the stock culture for all of the various trials.

Table IV gives the results of two trials made on August 26, 1924, and September 6, 1924. It will be noted that the optimum temperature range for conidial germination lies between 14° and 18°C., while slight germination was observed at 5°C. Low temperatures seem to retard germination and to reduce the number of zoospores. The maximum temperature for conidial germination has not been determined so definitely as the minimum temperature. In one trial, 44 percent of the conidia germinated after 2¼ hours at 30°C. but in another trial no germination of conidia was observed after 12 hours at 29°C.

At the extremes of the temperature range of conidial germination, the protoplasm either fails to divide at all or, dividing incompletely, emerges from the conidium enmass.

TABLE IV. THE INFLUENCE OF TEMPERATURE ON CONIDIAL GERMINATION

Trial	Temp. C.	Percent germination									Maximum percent germination
		1 hr.	1¼ hr.	1½ hr.	1¾ hr.	2 hr.	2¼ hr	2½ hr.	12 hr.	14 hr.	
1	29°	0	....	....	0	....	....	....	0	....	0
	21°	....	41	....	73	....	....	76	....	....	76
	18°	15	....	77	....	87	....	....	....	....	87
	15°	0	....	50	....	....	90	....	....	....	90
	10°	....	0	0	....	....	25	....	44	....	44
	7.5°	....	0	0	....	....	....	....	12	....	12
2	23°	40	....	40	....	....	....	....	....	....	40
	18°	42	....	55	....	....	....	....	....	....	55
	14°	....	40	....	55	....	....	....	....	....	55
	11°	28	....	33	....	....	....	....	....	....	33
3	30°	....	....	....	....	....	44	....	....	....	....
	4°	....	....	trace	....	....	....	2	....	....	.
	15°	....	....	71	....	....	....			..	....

At 7.5°C., a zoospore was seen to come out of a conidium, round up immediately and remain quiescent. At nearer optimum temperatures the zoospores are very active and remain so for a longer time than at less favorable temperatures. Normal zoospores are oblong; blunt at the forward end and taper posteriorly. The tail is pointed.

One of the cultures that had remained at 4°C. for 90 minutes and showed only traces of germination was changed to 15°C. A count was then made showing that 46 percent of the spores had germinated. Another culture having but 2 percent germination after 4 hours at 4°C., germinated 50 percent when put at 15°C.

LARGE CONIDIA

On August 26, 1924, several large conidia-like structures were found among conidia that were taken from a growing plant, which had been in a damp chamber 7½ hours. The leaves had been wiped clean of conidiophores. The large spore-like structures measured 43 x 18.6μ., while ordinary conidia measure 14-23 x 11-17μ.

Three days later other large, oblong spores were noted among conidia in a water culture. One apparently contained six quite distinct zoospores that had rounded off inside and had not been able to escape. Another large spore was partially filled by two zoospores while another was empty. These spores, similar to the conidia, were oblong and about two and one-half times larger than the others.

Again on September 4, a few large, oblong conidia were found among conidia from leaves which had been kept in a damp chamber all day. In this connection it should be noted that Shirai

(17) found two sizes of conidia of *Sclerospora graminicola*: 24-28.8 x 16.8-19.2μ and 38.4-57.6 x 19.2-24μ.

LENGTH OF CONIDIOPHORES

Leaves were prepared for conidiophore measurement by setting them on edge in small glass chambers made from two glass slides placed one above the other. These were sealed about the sides with beeswax and wet cotton was stuffed in at the ends. This arrangement made it favorable to study sporulation without exposing the leaves to the low humidity in the room. The leaves were placed on edge in order that the conidiophores would extend outward in a horizontal plane. Thus it was possible to measure them while still alive and turgid.

Twenty-nine mature conidiophores were examined. Measurement was made from the base of the conidiophore at the leaf surface to the outermost tip. The average length was 334μ. Six conidiophores just showing branch buds averaged 255μ.

Eleven measurements of conidiophores scraped from the leaves of *Setaria viridis* gave as follows:

	375.3μ.
	234.0μ.
	214.5μ.
	288.6μ.
	241.8μ.
	319.8μ.
	234.0μ.
	241.8μ.
	273.0μ.
	308.1μ.
	214.5μ.
11	2945.4μ.
	267.8μ. Average length.

Butler (2) gives the length of *Sclerospora graminicola* conidiophores as 100μ., Weston finds them to vary between 100 and 200μ., while Shirai (17) places their length between 100 and 240μ. It is obvious that the length is variable and that the range may be considerable. Differences as great as 160μ. are not uncommon.

DISCHARGE OF CONIDIA

On September 5, 1924, some infected leaves of *Setaria viridis* were washed and while still damp were incubated in a tumbler over night in the greenhouse. In the morning white spots were noted on the glass near some of the leaf surfaces. Microscopic examination revealed these white glistening spots to be great numbers of conidia.

To test further the discharge of conidia, a double glass slide was prepared as previously described. An infected leaf was inserted within the opening. The whole apparatus was placed

TABLE V. THE DISCHARGE OF CONIDIA FROM CONIDIOPHORES OF *SCLEROSPORA GRAMINICOLA*

Date	Direction of discharge	mm.
9/ 6/24	Vertically	1.5
9/ 6/24	Vertically	2.5
3/16/25	Horizontally	1.44
3/16/25	Horizontally	1.71
3/16/25	Horizontally	1.89

in a damp chamber to insure proper moisture conditions. Seven hours later, conidiophores could be seen in all stages of development. There were unbranched initials with stubs just arising from the stomata, while others were fully branched and bearing tiny conidia. At 8¾ hours the conidia appeared mature, but none was adhering to the glass above. After 10 hours many conidia had been discharged and were held to the upper slide by a film of condensed water.

The distance from the top of the conidiophores on the leaf to the majority of discharged conidia above was 1.5 mm. This was determined by focusing the microscope on the two points successively and measuring the difference in elevation of the scope. Those conidia which were thinly scattered on the glass were thought to have been discharged from the farthest points. A few conidia were found attached to the upper slide 2.5 mm. from the nearest conidiophore and this distance was considered the greatest that the spores were forced.

In another experiment conidiophores were observed extending horizontally outward from the leaf at a point near the glass slide beneath. On the slide were many discharged conidia. Measurements from the top of the conidiophores outward to the most distant conidia gave the distances enumerated in table V.

CONIDIAL INFECTION

A number of varieties of *Zea mays* (corn), *Holcus sorghum* (sorghum) and Setaria were planted in sterilized soil. On 9 and 11 days after planting the seedlings were sprayed with a conidial suspension. To insure more thoro wetting of the leaves, they were rubbed between the fingers and more inoculum was added. The plants were kept in a damp chamber over night.

Infection occurred only as noted in table VI on one corn, two teosinte, 30 Setaria and four plants of common millet. The percentage of infection was not as high as expected and emphasizes the evanescent nature and marked dependence of the organism on favorable moisture conditions.

Conidial sporulation appeared on the leaves of *Setaria viridis* six days after the young plants had been sprayed with a conidial water suspension. Conditions were made as favorable as possible for conidial fructification by placing the plants in moist

TABLE VI. THE RELATIVE RESPONSE OF A GROUP OF PLANTS WHEN EXPOSED TO CONIDIA

Plants exposed	Age in days	Total plants	Plants sporulating	Plants striped only	Percent infection
Holcus sorghum					
Sorghum (Red Amber)	11	Very many	0	0	0
Sorghum (Black Amber)	11	" "	0	0	0
Sorghum (Orange)	11	" "	0	0	0
Sorghum (Sumac)	11	28	0	0	0
Euchlaena mexicana (Teosinte)	11 and 9	40	1	2	7.5 .....
Zea mays					
Sweet corn (Golden Bantam)	11	9	0	0	0
Sweet corn (Narrow Grain Evergreen)	11	7	0	0	0
Sweet corn (Crosby)	11	9	0	0	0
Sweet corn (Cream and Honey)	11	5	0	0	0
Sweet corn (Black Beauty)	11	7	0	0	0
Pop corn (Japanese Hulless)	11 and 9	33	0	0	0 .....
Pop corn (Baby Golden)	11	7	0	0	0
Pop corn (Yellow Pearl)	9	17	0	0	0
Pop corn (May's Golden)	9	17	0	1	5.9
Dent corn (Silvermine)	9	12	0	0	0
Dent corn (Reid's Yellow)	9	4	0	0	0
Setaria viridis	9	300	30	.....	10
Setaria italica (Common millet)	9	34	4	.....	11.8
Setaria italica (Subsp.) (Siberian millet)	9	300	0	0	0

chambers. The fungus fruited on the sixth day; the same length of time required for fructification following oospore infection.

OOSPORE RESPONSE

The fact that it has been possible in these studies to obtain infection with the oospores of *Sclerospora graminicola* has made it possible to accumulate much information about their function and also their response to environmental conditions.

PRELIMINARY OOSPORE INFECTION EXPERIMENTS

From the evanescent nature of the conidia of *Sclerospora graminicola* it would seem highly improbable that the fungus is dependent upon these spores to carry it from one year to the next. Having had only fair results in producing the disease from conidia, attention was turned to the oospores to ascertain their ability to produce infection.

Twelve 5-inch pots were prepared with ordinary, unsterilized greenhouse soil on November 25, 1924. Oospores of *Sclerospora graminicola* were mixed in the upper 1½ inches of soil in all pots except the two for checks. Five pots were placed in the ground outdoors, while the rest were left in the greenhouse. The minimum temperature outdoors during the experiment was —21°F.

TABLE VII. A STUDY OF OVERWINTERING OF THE OOSPORES OF *SCLEROSPORA GRAMINICOLA*

No. of cultures	Treatment of pots containing oospores and soil	Setaria seeds planted	Pots restored to greenhouse	Percent plants infected
		1924	1925	
1	Held on greenhouse bench	Nov. 25	.....	1
2	Check. In greenhouse; no oospores	Nov. 25	.....	0
1	Sunk in ground outdoors	Nov. 25	Feb. 9	20
		1925		
1	Sunk in ground outdoors	Feb. 9	Feb. 9	20
2	Sunk in ground outdoors	Mar. 27	Mar. 27	70
1	Sunk in ground outdoors	May 27	May 27	2
1	Kept moist under greenhouse bench	Feb. 9	.....	20
2	Kept moist under greenhouse bench	Mar. 7	.....	15
1	Kept moist under greenhouse bench	May 27	.....	20

The pots kept in the open were brought into the greenhouse and the soil allowed to thaw.

As indicated in table VII these tests show a higher percentage of infection on *Setaria viridis* from oospores wintered outdoors than from those in the greenhouse. Every culture containing oospores produced some infection regardless of treatment, while the check developed none.

Similar results were obtained in a second trial involving 10 pots in which *Setaria viridis* was planted in sterilized and artificially infested soil. In every one of the 10 pots of soil infested with oospores, some plants were infected while in 5 other pots similarly treated, except not infested, no plants showed infection.

Results of these tests show that seedlings of *Setaria viridis* may be readily infected by oospores of Sclerospora in soil artificially infested and held outside or inside the greenhouse.

OOSPORES OVER-WINTERING IN FIELD SOIL

A question still remained regarding the method by which *Sclerospora graminicola* lives thru the winter in nature. To determine this, a quantity of frozen field soil known to contain oospores was taken into the laboratory in February and March. After the soil had thawed, it was potted and all except seven pots were planted with the seeds of various hosts. In all of these pots the volunteer Setaria plants were allowed to grow.

As shown in table VIII, in 20 of the 22 pots of untreated field soil the plants developed infection, while plants grown in the same soil after sterilization were free from infection. Repeated trials using sterile soil gave no evidence to support the possibility of the fungus over-wintering in the mycelial stage within the seeds of host plants.

A more elaborate over-wintering test was conducted during the winter of 1926-27. Oospores collected in September, 1925, were compared with others collected in September, 1926. White sand, greenhouse potting soil and black loam taken from a corn

TABLE VIII. INFECTION PRODUCED IN FIELD AND GREENHOUSE SOILS NOT ARTIFICIALLY INFESTED WITH OOSPORES

No. of pots of plants exposed	Source of soil used	Soil treatment	Plants grown	Plants infected
2	Field	Sterilized	*Setaria viridis*	0
2	"	Untreated	*Setaria viridis*	2
7	"	"	*Setaria viridis* (volunteers only)	9
2	"	"	*Setaria italica* (millet)	1
2	"	"	*Setaria viridis*	1
2	"	"	*Setaria italica* subsp. (Siberian millet)	0
2	"	"	*Setaria italica* subsp. (White wonder millet)	1
5	"	"	*Zea mays* Sweet Corn (Golden bantam)	0
		"	*Setaria viridis* (volunteers)	17
1	Greenhouse	"	*Setaria viridis*	0
		"	*Setaria italica* (millet)	0
1	"	"	*Setaria italica* subsp. (Siberian millet)	0
1	"	"	*Setaria italica* subsp. (White wonder millet)	0

field south of Ames, Iowa, were also compared as to their effect on the vitality of the oospores. The oospores were thoroly mixed with the soil in test tubes. Half of the cultures were wet with tap water, while the others were left dry. Cotton was used to plug the tubes. The series was placed in a large greenhouse pot and lowered in the ground so that the soil-oospore mixtures in the tubes were 8 inches below the surface. Field soil was packed firmly about the tubes and smoothed over the top. This was done October 14, 1926, and the tubes were taken up in

Fig. 1. A plant of popcorn (Japanese Hulless) infected with *Sclerospora graminicola*. It was planted on March 31, 1927, and photographed 64 days later. Note the extreme shortening of the internodes which has caused the overlapping of the leaf sheaths on the one side and which has spread them far apart on the other. The transparency of the leaf veins imparts a streaked appearance to the leaves, while the small chlorotic areas between the veins give the typical mottled effect. The plant measures 7 inches in length.

TABLE IX. OVER-WINTERING OF OOSPORES IN THE SOIL

No.	Time oospores collected	Soil	Condition of spores	Physical condition when raised	Seed exposed	Total plants exposed to infection	Percent Infected
1	1925	Sand	Wet	Damp	*Setaria*	14	43
					Zea mays	3	33
2	1926	"	"	Water standing	*Setaria*	25	8
					Zea mays	4	0
3	1925	"	Dry	Damp	*Setaria*	35	31
					Zea mays	3	33
4	1926	"	"	Dry to damp	*Setaria*	35	29
					Zea mays	2	0
5	1925	Potting	Wet	Very wet	*Setaria*	33	18
					Zea mays	2	0
6	1926	"	"	Water standing	*Setaria*	28	23
					Zea mays	2	50
7	1925	"	Dry	Moist	*Setaria*	30	57
					Zea mays	4	50
8	1926	"	"	"	*Setaria*	41	27
					Zea mays	3	0
9	1925	Black loam	Wet	Sticky	*Setaria*	35	34
					Zea mays	2	0
10	1926	" "	"	"	*Setaria*	20	20
					Zea mays	3	0
11	1925	" "	Dry	Dry	*Setaria*	28	18
					Zea mays	3	33
12	1926	" "	"	"	*Setaria*	14	36
					Zea mays	4	0
13	Mixed	Potting	Moist	Wet	*Setaria*	30	17
					Zea mays	3	0
14	Mixed	Sand	"	Moist on top	*Setaria*	12	42
					Zea mays	4	25
15	Mixed	Black loam	"	" "	*Setaria*	15	13
					Zea mays	4	25
16	1926	Sand	Ck.	Kept in lab.	*Setaria*	2	0
					Zea mays	2	50
17	1926	Potting	"	" "	*Setaria*	41	29
					Zea mays	9	67
18	1926	Black loam	"	" "	*Setaria*	4	25
					Zea mays	2	100
19	1925	Sand	"	" "	*Setaria*	22	0
					Zea mays	1	0
20	1925	Potting	"	" "	*Setaria*	169	14
					Zea mays	4	25
21	1925	Black loam	"	" "	*Setaria*	.....	.....
					Zea mays	2	0
22	Ck.	Potting	No oospores	No oospores	*Setaria*	43	0
					Zea mays	5	0

April, 1927. The physical condition of the soil was also recorded. Both popcorn (Japanese Hulless) and *Setaria viridis* were planted in the soil from the tubes.

In table IX are given the results of over-wintering of oospores in the soil. Every culture infested with oospores, except No. 21, produced some infection. Fig. 1 shows a diseased plant of popcorn (Japanese Hulless) produced in this experiment. This indicates that oospores remained alive in nearly every case. In these experiments, the percentage of infection caused by spores over-wintering outdoors was nearly twice that caused by spores held in the laboratory during the same period. The types of soil in which the spores were wintered did not seem to influence the ability of the spores to produce infection. Wet or dry spores produced about the same percentage of infection.

HOSTS OF SCLEROSPORA GRAMINICOLA*

This fungus is most familiarly known in the United States on *Setaria viridis* (L.) Beauv. Altho Sclerospora has been reported on *S. glauca* (L.) Beauv. in Europe by Schroeter (16) and in this country by Wilson (27) and by Davis (5), it has never been collected by the authors and all inoculations have failed. This is rather significant since *S. viridis* and *S. glauca* are two annual species commonly found growing together in cultivated soil in Iowa. *S. viridis* (L.) Beauv., usually known as foxtail or pigeon grass, has a pointed head and straight flat or untwisted blades, while *S. glauca* (L.) Beauv. (yellow foxtail) has yellow obtuse heads, the blades being so twisted that the upper surface at the end is underneath.

Butler (2) describes the attack of Sclerospora upon *Setaria*

TABLE X. SUSCEPTIBLE HOSTS OF *SCLEROSPORA GRAMINICOLA*

Plants exposed to infection	Number	Percent plants infected
Euchlaena mexicana (teosinte)	Many	43
Setaria viridis	"	90
Setaria (millet)		
White French	"	15
Siberian	"	30
Common	"	70
White Wonder	"	15
Hungarian	"	30
Holcus sorghum (sorghum)		
Red Amber	"	3
Black amber	"	2
Orange	"	5
Sumac	"	2
Saccharum officinarum (cane)		
Black Amber	"	7
Orange	"	10.5
Zea mays (popcorn)		
Baby Golden	5	20
Yellow	8	37
Black Beauty	6	17
Japanese Hulless	7	70
American Wonder	10	10
Yellow Pearl	10	70
May's Golden	8	75
Zea mays (sweet corn)		
Golden Bantam	32	9
Golden Dawn	3	33
Narrow Grain Evergreen	7	14
Crosby	7	14
Golden Giant	6	33
Zea mays (dent corn)		
Silver King	5	20
Reid's Yellow Dent	14	21
Silvermine	7	14
Iajap Striped	7	14
Hardin County White	6	33
MacArthur's Golden King	6	17
Walden Dent	7	29
Ioleaming	7	14
Edward's White King	7	29
Golden Jewell	7	14
Iowanda	7	43
Golden Murdock	7	29
Workman	7	29
King Yellow Victor	7	29
Iowa 119	7	29

*See note at bottom of page 298.

italica, Andropogon sorghum Brot., *Euchlaena mexicana* Schrad. (teosinte) and *Pennisetum typhoideum* Rich. (pearl millet). Kulkarni considers the fungus which attacks sorghum (jowar) to be a subspecies of *Sclerospora graminicola.* His reason is based upon the fact that the conidia of the sorghum mildew germinate by tubes instead of producing zoospores as is typical with *S. graminicola.*

Melhus and Van Haltern (10) first reported Sclerospora on corn in the United States. More recently Weston and Weber (25) have described the mildew of *Setaria magna* in the everglades of Florida.

Table X gives a list of plants that were found to be susceptible in studies of the host range of Sclerospora among economic plants. Other plants exposed to infection which developed no symptoms of infection were: *Setaria glauca, Panicum miliaceum* L. (broom corn millet), *Setaria italica* (Japenese millet), *Pennisetum typhoideum* Rich. (pearl millet), *Holcus sorghum* (red kafir), *H. sorghum* (white kafir), *Zea mays* (White Rice popcorn) and six other varieties of *Zea mays* (sweet corn): Cream and Honey, Bantam Evergreen, Golden Evergreen, Country Gentleman, Golden Country Gentleman and Seymours Sweet Orange. However, it is not improbable that in further trials some or all of these hosts might become infected. Oospores which had been threshed from the shredded heads and leaves of *Setaria viridis* (see fig. 2) were sprinkled over the seeds at the time of planting.

Fig. 2. Oospores of *Sclerospora graminicola* are obtained from such diseased plants of *Setaria viridis* as "A" and "C" of this picture. Vast numbers of oogonia form within the diseased tissues of the leaves and heads during the latter part of the summer. The infested parts often exhibit strange abnormalities of form. Finally, the diseased tissues are killed and disintegrate to such an extent that only the vascular elements remain. The infested heads of plant "C" may be compared with the normal head "B."

In the case of corn it was found that seedlings were more apt to become infected with the mildew if the seeds were placed on the seedbed, germ-side up, and if a small bunch of oospores was dropped on each germ. Other oospores were then mixed with the top soil. To lessen the danger of the oospores being washed away from the seeds when the bed was watered, the seedbed was wet down before the seeds were planted. Then, if the pots needed water before the plants appeared above the ground, it was supplied by capillary attraction from below. By using such precautions it was quite certain that the developing plumule of each seedling would be in physical contact with oospores as soon as the plumule ruptured the seed coat. Fig. 3 pictures two plants of popcorn (Golden Bantam) which were exposed to oospores under the above conditions. The larger plant is healthy while the stunted one is diseased.

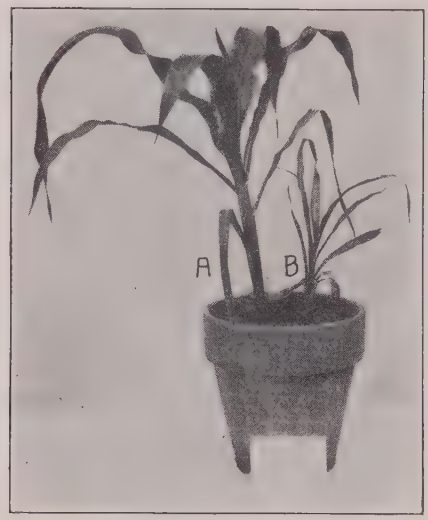

Fig. 3. Golden Bantam is one of the varieties of sweet corn most susceptible to infection by *Sclerospora graminicola*. These plants came from seeds which were exposed to infection by oospores in the soil. When the first leaves unrolled it was evident from the irregular, chlorotic mottling of its leaf blades that "B" was diseased. At first the two seedlings were much alike in form, but later the healthy plant "A" greatly outgrew its companion. Only slight elongation of the internodes took place in plant "B" and thus caused its stunting. The normal leaves are broad and gracefully curved, while those of the diseased plant are narrow and rise stiffly from the sheath.

The manifestation of Sclerospora as a seedling blight of corn is pictured in fig. 4. Oospores were placed in the four pots at "A" while the remaining pots at "B" were held as controls without oospores.

Plants from five genera of Gramineae were found to be susceptible hosts to *Sclerospora graminicola*. These are, as shown in table X, Euchlaena, Setaria, Holcus, Saccharum and Zea. *Setaria viridis* was found to be most susceptible of all the plants exposed. Of the three races of Zea (popcorn, sweet corn and dent corn) exposed to infection by oospores, popcorn was the most susceptible while dent corn appeared least susceptible. Teosinte ranked next to popcorn in percentage of infection produced. Of 11 varieties of sweet corn exposed, only 4 were sus-

Fig. 4. The effect of *Sclerospora graminicola* in producing seedling blight of corn is well illustrated in this test with popcorn (May's Golden). The plants at "B" were grown in pots in the greenhouse but were not exposed to infection by oospores. A like number of seeds were planted in the pots at "A" and given identical cultural treatment, but these were exposed to large numbers of oospores in the soil. The few plants that were not killed outright are shown to be stunted by the disease. Only two plants are not infected.

ceptible, but it is probable that further trials will prove the others susceptible also.

Altho Butler (2) reports damage to *Pennisetum typhoideum* by *Sclerospora graminicola* in India, no infection so far has been obtained here, either in the greenhouse or field.

Data on the incubation period for oospore infection are presented for the first time. The duration of this period involved the time between exposing the seeds to oospores and the beginning of conidial fructification. As this organism does not fruit readily, except in a very humid atmosphere, all the cultures were

TABLE XI. THE INCUBATION PERIOD FOR INFECTION WITH OOSPORES

No.	Host	Date exposed	Substrate used	Place incubated	No. days before conidia appeared	No. days before mottling appeared
		1925				
1	*Setaria viridis*	Mar. 3	Sterile soil	Damp chamber	6	....
2	" "	Mar. 14	Pfeffer's agar	Greenhouse	7	....
3	" "	Mar. 15	Sterile soil	Greenhouse	6	....
4	" "	Mar. 15	Pfeffer's agar	Damp chamber	6	....
5	Popcorn (Jap. hulless)	Apr. 14	Sterile soil	Greenhouse	....	10
6	Common millet	Apr. 18	Sterile soil	Greenhouse	6	....
7	*Setaria viridis*	Apr. 18	Sterile soil	Greenhouse	6	....
8	Popcorn (Jap. hulless)	Apr. 18	Sterile soil	Greenhouse	....	6
9	Teosinte	Apr. 18	Sterile soil	Greenhouse	....	6
10	Popcorn (May's golden)	Apr. 18	Sterile soil	Greenhouse	....	8
11	Popcorn (May's golden)	May 17	Sterile soil	Greenhouse	....	10

placed in a moist chamber, the walls and ceiling of which were of glass, lined with cheese cloth. A 3-inch layer of wet sphagnum moss covered the floor. The moist chamber was kept in a "head house" off from the greenhouse where a temperature of 15° to 20°C. was maintained. Before placing plants in the chamber, the walls and ceiling were thoroly sprayed with water. Then the plants were inserted and the air completely saturated with moisture by the use of an atomizer. Usually this was done in the evening in order to take advantage of the lower temperatures. The plants were comparatively warm at first but as they cooled a film of moisture was deposited over the leaves by the surrounding water-saturated air.

Several trials are recorded in table XI in which the exposed plants were held continuously in the moist chamber. This was done to enable the mildew to develop its conidiophores at the earliest possible time. In other tests the plants were kept on the greenhouse bench until the first leaves had unrolled.

Usually, the period of incubation from the time the oospores were applied to the seeds to the production of the first conidia was six days. This is the same period of time required for conidial infection.

If *Sclerospora graminicola* is not present in the seeds of corn the mycelium must enter the seedling and grow into the leaves before systemic infection is evident. Often the pale yellow blotching on the leaves is found on the first leaf above the coleoptile 6 to 10 days after the seeds are planted. Infection occurs in a similar manner on *Setaria viridis*, tho usually in a

TABLE XII. THE RELATION OF AGE OF POPCORN SEEDLINGS AND SUSCEPTIBILITY TO OOSPORE INFECTION

Exp. No.	Host	Date planted	Interval between planting and exposing seedlings to oospores	Size when exposed		Total No. plants exposed	Percent infected
				Radicle	Plumule		
		1925					
1	Popcorn (Japanese Hulless)	Apr. 2	0 days	.....	.....	18	44
	" "	"	1 "	Just showing	.....	9	22
	" "	"	2 days	¼ to 1 in.	¼ to ½ in.	9	0
	" "	"	4 "	1½ to 2 in.	½ to 1 in.	9	0
	" "	"	6 "	.....	1½ to 2 in.	11	0
	" "	"	Ck: Seedlings not exposed	.....	.....	18	0
2	Popcorn (Yellow Pearl	Apr. 20	0 days	.....	.....	6	67
	" "	"	1 "	.....	.....	10	80
	" "	"	2 "	Just starting	Just starting	9	22
	" "	"	3 "	0 to 1 in.	¼ in.	8	25
	" "	"	4 "	1 to 3 in.	¼ to ½ in.	1	0
	" "	"	6 "	Long	About 1 in.	10	10
	" "	"	Ck: Seedlings not exposed	.....	.....	13	0

TABLE XIII. THE RELATION OF AGE OF POPCORN SEEDLINGS AND SUSCEPTIBILITY TO OOSPORE INFECTION

Exp. No.	Host	Date seeds planted and exposed	Interval between exposing seeds and removing oospores by washing	Size when washed		Total plants	Per-cent infected
				Radicle	Plumule		
3	Popcorn (Yellow Pearl)	1925 Apr. 20 Ck. not exposed				9	0
	" "	Apr. 20	1 days			8	0
	" "	"	2 "			8	25
	" "	"	3 "			9	33
	" "	"	4 "	0 to ¾ in.		9	56
	" "	"	5 "	1 in.	0 to ½ in.	13	0
	" "	"	Full period	Long	¼ to 1 in.	19	32

shorter time. In the latter, not only do the leaves become etiolated, but it is not uncommon to have conidiophores developed six days after the seeds are planted.

In other inoculation trials with oospores, the incubation period for corn, as determined by conidial production, has been as long as 23 days. For *Setaria viridis* the period seemingly depends upon the rapidity with which the cotyledon develops. Under normal conditions this is seldom longer than 10 days.

THE RELATION OF AGE OF POPCORN SEEDLINGS AND SUSCEPTIBILITY TO INFECTION BY OOSPORES

In an effort to determine the stage in germinating corn at which oospore infection takes place, a number of pots of sterilized soil were prepared and planted with popcorn (Japanese Hulless). The seeds in the first pot were exposed to oospores at the time of planting. At intervals from one to six days, the plants were removed and oospores were heavily dusted over seeds, plumules and roots after which the soil was restored to its place. From the data presented in table XII, experiment 1, it is suggested that infection takes place only in the very early stages of germination.

In order to test further the relationship between age and infection by oospores, a set of pots were prepared as before. This series was started April 20, as shown in table XII, experiment 2, and the experimental procedure was carried out in the same manner as for the preliminary experiment, table XII, experiment 1. In experiment 3, table XIII, all seeds were exposed at the time of planting by placing oospores on each seed. After 24 hours the germinating seeds from one of the pots were taken up and freed as far as possible from all adhering oospores by washing in running tap water. Considerable time was consumed in the operation for the seedlings were frequently examined microscopically to see that no oospores remained attached. The washed seedlings were then replanted in a fresh pot of sterile

TABLE XIV. METHODS OF EXPOSING SEEDLINGS TO INFECTION

No.	Host	Position of oospores in culture pots	Treatment	Water added from	No. plants exposed	Percent plants infected
1	Popcorn (Yellow Pearl)	Layer ¼ in. below seeds	.....	Bottom	10	10
2	" "	Just below seeds	Seeds germ-side up	"	8	13
3	" "	Seeds covered	" "	"	7	86
4	" "	Dusted on seeds	Only spores clinging to seeds left	"	4	25
5	" "	Mixed with top soil	.....	"	4	50
6	" "	Layer ½ in. above seeds	.....	"	8	0
7	" "	Layer on soil surface	Covered with damp cloth	"	9	11
8	" "	" " "	Not covered	"	10	0
9	" "	" " "	" "	Top	9	11

soil. At intervals of 24 hours the remaining seedlings were taken up, washed and repotted in new sterile soil.

On April 22 (table XIII, experiment 3) five seeds were just starting to germinate at the time the oospores were removed. Later two of these proved to be infected. This would indicate that the sprouts were infected immediately after the pericarp was ruptured. Seedlings exposed to the spores for longer periods showed higher percentages of disease.

A third test was devised to determine more definitely the time of seedling infection. Four-inch pots containing sterile soil were prepared so that the oospores, when added, lay at various levels either above or below the seed. By this arrangement the growing seedlings would come in contact with the spores at different stages in their development. In pot No. 1, the layer of spores was placed ¼ inch below the seeds; in the second pot, the seeds were placed germ-side up on a layer of spores; in pots 3 and 4, the oospores were applied directly to the seeds, etc.

Results given in table XIV indicate that the greatest amount of infection can be brought about by covering the seeds with oospores before adding the top soil. Moisture seems necessary for oospore germination as shown in cultures 7 and 8. When the surface layer of spores was covered with a damp cloth, 11 percent infection occurred (No. 7). When no protection was given the spores (No. 8), none of the 10 plants became diseased. To guard against washing the spores down to the seed, the first eight pots were watered by setting them in a shallow pan of water. In the first pot the spores were either carried upward to the seeds by the capillary movement of the water, or infection occurred when the radicle penetrated down to the oospores. In the case of pot 9, where top watering was practiced, it is probable that some of the surface spores were washed into the soil.

Another experiment, to ascertain at what stage corn seed-

TABLE XV. THE RELATION OF SEEDLING DEVELOPMENT AND SUSCEPTIBILITY

No.	Interval from time of exposing to emergence of plumule above ground (days)	Total No. plants exposed	Percent. plants infected	*Number* of plants infected and *days* elapsing from plumule emergence to first appearance of symptoms																	
				Days																	
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	Check:*	31	77.4	...	...	...	10	9	2	1	2	....	....	....	....	....	....	....	....	....	....	....	....
1	10	1	0	...	...	...	...	...	...	...	...	....	....	....	....	....	....	....	....	....	....	....	....
2	9	7	57.1	...	...	...	1	1	...	1	...	....	....	....	....	....	....	....	....	....	....	....	....
3	8	2	100.0	...	...	...	1	1	...	...	...	....	....	....	....	....	....	....	....	....	....	....	....
4	7	23	65.2	...	...	...	4	1	3	6	...	....	....	1	....	....	....	....	....	....	....	....	....
5	6	20	70.0	...	...	...	1	5	1	1	2	....	1	1	....	....	1	1	....	....	....	....	....
6	5	16	43.7	...	...	...	...	1	...	2	1	2	....	1	....	....	....	....	....	....	....	....	....
7	4	13	50.0	...	1	...	...	...	1	...	...	3	....	....	....	2	1	....	1	....	....	....	....
8	3	51	39.2	...	...	...	...	...	...	3	2	2	2	....	1	....	1	2	....	2	....	5	2
9	2	60	30.5	...	...	...	...	...	1	...	1	2	1	1	....	....	2	2	4	....	2	....	4
10	1	38	34.2	...	...	...	...	...	...	...	....	1	1	1	1	....	....	....	2	2	....	2	1
11	0	9	33.3	....	...	....	....	....	....	....	....	....	....	....	....	....	....	....	1	....	1	....	1
Totals		345																					

*Seedlings exposed to infection when planted.

lings are susceptible and whether pre-soaking of oospores influences infection, was conducted with two sets of pots containing sterile soil and planted with popcorn (Yellow Pearl). As a check, the soil in four pots was infested with oospores at the time of planting. The soil of the remaining pots was infested on successive days up to the time the plumules appeared above ground. The oospores used in this experiment were divided into two lots. The first lot was mixed with sterile soil at the time the seeds were planted. The oospore-soil mixture was kept in a covered receptacle in the greenhouse under identical temperature conditions with the seedlings. The other lot of oospores was kept dry in the laboratory until the oospores were used for infection studies.

Each plant in this experiment was labeled and data on inoculation and appearance of disease symptoms were recorded. By means of transplanting, an effort was made to have all plants in each pot of uniform size.

A comparison of the centages of infection obtained by the use of dry oospores showed no significant differences. The average percentage of infec-

tion with dry spores was 45.6 while that with the wet spores was 42.5.

Table XV gives all the data of this experiment without regard to oospore treatment. This is possible because the procedure with both was the same with the exception of spore treatment.

In culture pot 11, three out of nine seedlings became infected as a result of being inoculated just as the plumules appeared at the surface. This indicates that the oospores either germinate very quickly after being placed in the soil or that infection may occur over a longer period of time than previous experiments had shown.

The data in table XV reveal a rather striking distribution. Those plants exposed 10 days before the emergence of their plumules above ground gave the earliest evidences of infection, while those exposed later became less infected. It will be seen, however, that the intervals between the time of exposure of the plants and the time infection became evident are about equal. Early trials gave higher degrees of infection than those made later. This correlates with data presented in table XII. It is also to be noted that early infection seems to be more destructive to corn.

SEEDLING STRUCTURES SUSCEPTIBLE TO OOSPORE INFECTION

After securing the above data regarding the stage in seedling growth most susceptible to oospore infection, a number of tests were devised with the hope of ascertaining the place where the fungus enters the host. This was done as follows: quantities of oospores were brought in contact with the leaves, roots and the plumules of growing seedlings. Any infection resulting was apparently produced thru the part exposed.

Six pots containing *Setaria viridis* in the three-to-four-leaf stage, were selected. After moistening the leaves with water applied with an atomizer, oospores were dusted on quite heavily and the leaves were lightly sprayed again. The pots were placed in the moist chamber over night. No infection developed on any of the plants altho they were kept under observation for three or four weeks.

To check the vitality of the oospores used in this test, other spores from the same collection were placed on seeds in the soil by the usual method and infection was obtained in all cases.

In order to insure enough moisture being present in the first trial to produce infection, a second series of *Setaria viridis* plants was exposed to infection as described above and kept in the moist chamber continuously for 39 hours. The leaves were wet all of the time. One of the pots, after being out of the moist chamber for a day, was returned for an additional three-day period.

No sign of infection could be detected 12 days after the plants

were exposed. This suggests that the germinating oospore is not able to penetrate older leaf tissue, but this phase of the problem needs further study.

Attention was then turned to the possibility of infecting the radicle or roots. Sprouting kernels of popcorn (May's Golden) were suspended between the points of bent wires about 3/16 of an inch above a 200 mm. culture dish containing a mixture of sterile soil and Sclerospora oospores. At the same time, 10 kernels were planted in a similar dish containing sterile soil and oospores. To supply the seedlings with sufficient moisture, the dishes were covered until the growing plumules became too tall. The radicles of most of the plants found their way into the infested soil but very few secondary roots reached it. Of the 12 seedlings suspended above the soil, none became infected. Of the 10 seedlings planted in the second dish, 5 were diseased.

In a second trial, popcorn (Japanese Hulless) was germinated in sterile sand. At the time the plumules had reached a length of 1/16 to 1 inch, the radicles were inserted thru holes in a cheese cloth. The cloth was then placed on a layer of heavily infested soil in a 2-gallon crock. In this way only the roots came in contact with the soil.

Other seedlings, of the same lot, were placed on a galvanized window screen which was suspended a little above heavily infested soil in a second container. As before, few secondary roots reached the soil.

None of the 24 seedlings growing thru cheese cloth contracted the disease. Infection of the 23 plants suspended on the wire screen was likewise negative. Of 15 check seedlings set in similarly infested soil, 2 became infected.

Thus, out of 59 plants whose roots were growing into heavily infested soil, none became infected. At the same time, 7 of the 25 check plants contracted the disease.

THE RELATION OF TEMPERATURE TO INFECTION BY OOSPORES

The relation of environment to conidial sporulation has already been discussed. It was pointed out that somewhat low temperatures in connection with moisture films on the leaves are the most important factors influencing the emergence of the conidiophores from the substomatal cavities. Also, as was shown in table IV, the conidia prefer about the same temperatures for germination, 15° to 20°C.

Germination of oospores has not been observed directly. The only available index to their optimal germination temperature, therefore, is obtained in the results of inoculations at different temperatures. For this purpose 20 4-inch pots and eight 1-quart cans of sterile soil were planted with popcorn (May's Golden). The seeds were covered with soil previously infested with oospores of *Sclerospora graminicola*. The cultures were

TABLE XVI. THE RELATION OF TEMPERATURE TO INFECTION BY OOSPORES

Corn variety	Place of germination	Ave. temperature			Incubation time (days)	Total plants incubated	Percent plants infected
		Air (deg.)	Soil (deg.)	Incubator water bath (deg.)			
Popcorn (May's Golden)	Incubator	36.4	30.1	.....	2½	103	3.8
Popcorn (May's Golden)	Greenhouse	25.0	24.1	.....	4	95	35.7
Popcorn (May's Golden)	Incubator	25.0	15.8	16.5	7	73	41.0

divided into three groups. Ten pots were placed on the greenhouse bench, 10 pots installed in an incubator and the 8 cans were set in a soil temperature control tank in the greenhouse. It should be noted that the temperature of the water in the tank on the last two days was high, ranging from 15.8° to 26°C.

At an average soil temperature of 30.1°C., 3.8 percent of the 103 plants exposed became infected. At a soil temperature which averaged 24.1°C., but which fluctuated between 16° and 33°C., 35.7 percent of the 95 plants exposed became infected. In the third location the soil temperature averaged 15.8°C. with little fluctuation except on the two nights mentioned. In the cool soil, infection rose to 41 percent of the 73 plants exposed. These data indicate that germination of oospores, like that of conidia, is favored by relatively low temperatures.

When considering the rapidity of germination of the corn at the highest temperature (two and one-half days from planting to emergence of plumule) one might think the time too short for the oospores to germinate and infect the host. This does not seem probable for in a previous experiment (table XVI), infection was induced after the plumule had reached the surface of the soil. If corn seedlings grown at low temperatures are more susceptible to this infection, then the conclusion on oospore germ-

TABLE XVII. THE EFFECT OF TEMPERATURE ON THE VIABILITY OF OOSPORES

Treatment of flats during germination	Treatment of oospores before placing them on the seed								Percent plants infected
	Check: No treatment		Dry heated 30° C. for 30 min.		Dry heated 84°C. for 30 min.		Frozen in water 30 min.		
	Plants exposed	Number infected	Plants exposed	Number infected	Plants exposed	Number infected	Plants exposed	Number infected	
On greenhouse bench continuously. Temp. 15° to 25° C.	24	1	24	1	22	1	0	....	4.3
Outdoors continuously. Temp. 0° to 20° C.	9	1	9	1	10	0	9	2	10.8
Days, outdoors; nights greenhouse. Temp. 10° to 20° C.	16	3	9	3	7	1	22	2	16.6
Percent of plants infected	....	10	....	12	....	5	....	13	.....

ination temperature may be wrong. However, it would seem that the rapidly growing seedlings would be more succulent and therefore infected with greater ease.

In a second test, oospores were divided into four lots. The first lot was held as a check, the second was dry heated at 30°C. for 30 minutes, the third was dry heated at 84°C. for 30 minutes, while the remaining spores were frozen in water for 30 minutes and subsequently thawed. Spores from each of the four lots were used to expose seeds of popcorn (Japanese Hulless). Two replications of the first planting were made. There were then three similar sets of two flats each.

The first set of flats was placed on the greenhouse bench where the temperature fluctuated between 15° and 25°C. during the germination of the seeds. The second set was placed in a partly sheltered position outdoors where the temperature range was 0° to 20°C. The remaining set of flats was carried outside each morning and returned to the greenhouse bench each night. Soil temperatures during the germination of these seeds varied between 10° and 20°C. Seven to 24 plants were produced in each series with the exception of one which was destroyed.

Table XVII shows that the temperature range of 10° to 20°C. was most favorable for oospore infection, while the higher one, 15° to 25°C., was least favorable. On the basis of infection obtained, there was no effect on oospores of dry heat at 30°C. for 30 minutes or freezing in water for half an hour. However, spores dry heated at 84°C. for 30 minutes, as compared with the others, produced less than half as many infected plants. This may indicate a weakening of the spores due to the heating.

THE RELATION OF VIABILITY OF OOSPORES TO TREATMENT WITH CHEMICALS AND HEAT

Biologic response of Sclerospora oospores to treatments with chemicals and heat is important since the oospores overwinter in the field soil. Neither water cultures nor media present adequate means of study because bacteria and protozoa usually obscure the oospores. Consequently, a study of the relation of viability of oospores to chemical treatment and heat was undertaken. The chemicals used were: sodium hydroxide, hydrochloric acid, formaldehyde, copper sulfate and mercuric chloride. Concentrations of 1, 2, 5 and 10 percent were used.

Most of the plants were killed when watered with the solutions so that the results are not complete. However, it is noteworthy that the 2 percent copper sulfate solution had little effect in decreasing infection from oospores in the soil. Oospores were killed by soaking in 2 percent copper sulfate solutions in treatments of more than 10 minutes duration. Infection was secured in another trial using 5 percent copper sulfate for 10 minutes.

No infection was obtained from oospores which had been

Fig. 5. Only in field tests can the true significance of the downy mildew disease of corn be judged. This is the manner in which popcorn (Japanese Hulless) developed in a test plot where the soil had been artificially infested with oospores. Ears are seldom produced on diseased plants like the three in the center of the picture. The tall plants are not affected by the mildews.

soaked in 1 percent solutions of formaldehyde during periods ranging from 5 minutes to 2 hours. Untreated spores produced 75 percent infection.

Mercuric chloride 1-1000 did not destroy the viability of oospores as readily as did the formaldehyde altho no attempt was made to compare their killing properties more fully.

Heating at 50°C. for 1 hour produced no weakening effects upon dry oospores. Wet spores, however, lost their viability to a marked degree under similar treatment. Dry heating for 1 hour at temperatures between 62° and 86°C. proved fatal to a large percentage of oospores altho in the case of newly collected spores, a few were still viable after being held at 76°C. for 3½

hours. It is noteworthy that oospores gradually lose their resistance to dry heat with age. Oospores collected in 1925 and heated for one hour at 77°C. produced no infection, while oospores of 1926 given similar treatment produced 52 percent infection on *Setaria viridis*. In the light of these tests, it is evident that oospores can be killed easily by use of present-day methods of soil sterilization.

Fig. 6. In Iowa conidial fructification of *Sclerospora graminicola* is often found occurring spontaneously on *Setaria viridis* in the field. With corn this condition is comparatively rare. These plants (Japanese Hulless popcorn) were grown in oospore-infested soil in the greenhouse, and the one shown at the center became infected. No conidia were found on the surface of the leaves until the plant had been in a moist chamber for eight hours in a saturated atmosphere at 18°C. Conidia were produced naturally on corn seedlings in field trials in 1926 and 1927 but only during periods of high humidity and cool temperature.

FIELD STUDIES OF SCLEROSPORA GRAMINICOLA

Laboratory and greenhouse experimentation is not complete without additional studies in the field. Consequently such investigations were made during the summers of 1925, 1926 and 1927.

Corn and teosinte

In June, 1925, popcorn (Yellow Pearl) was planted in a number of 2-inch paper pots of soil previously sterilized and infested with oospores. The pots were retained in the greenhouse until the plumules were beginning to push thru the surface. At that time they were carried to the field and transplanted. Of the 103 plants exposed, 26 became infected. None of the plants died in the process of transplanting, but the infected plants later died one after another until only 11 remained alive at earing time. Of the 93 check plants transplanted all lived except one.

On May 19, 1926, an experimental plot was planted with popcorn (Japanese Hulless). A pinch of oospores was placed on each seed before it was covered. Of the 132 plants in the plot, 30 became infected (see fig. 5) and 16 of these produced conidiophores on the leaves, showing clearly that infection would re-

Fig. 7. This hill of corn shows one normal stalk and one infected with *Sclerospora graminicola*. This popcorn (Japanese Hulless) was planted in May, 1927, in a field plot which was artificially infested with oospores. Thirty-six percent of these plants became diseased. A number of plants died in the seedling stage, while the rest became stunted and unproductive.

Fig. 8. In Iowa *Sclerospora graminicola* has been found occurring naturally on corn in the field only twice. This sweet corn (Golden Bantam) was discovered near Story City, August 15, 1927. The shortening of the internodes and the stiff, abruptly ascending leaves are two prominent symptoms of the disease. The tip of the tallest tassel measures 15 inches from the soil.

sult under field conditions where oospores were placed on the seed.

Fig. 6 pictures conidial fructification on popcorn (Japanese Hulless) planted at the same time in the greenhouse and subjected to optimum conditions for the development of the mildew.

Dent corn (Iodent), popcorn (Japanese Hulless), sweet corn (Golden Bantam and Evergreen) and *Euchlaena mexicana* (teosinte) were used in 1927 tests. The seeds were placed 3 inches apart in a furrow of moist soil 4 inches deep. After a pinch of oospores was placed on each kernel, a quantity of moist oospore-infested sand was scattered over the seeds and oospores and the top soil replaced. Twenty-four days after the plumules appeared above ground, the test plants were found to be infected as follows: Dent corn (Iodent) 13 percent, popcorn (Japanese Hulless) 36 percent, sweet corn (Golden Bantam) 19 percent, sweet corn (Evergreen) 19 percent and teosinte 8 percent.

Another test was made to determine the effect of temperature on the infection of popcorn (Japanese Hulless) germinated in greenhouse soil, heavily infested wtih oospores and later transplanted to the field. The amount of infection was determined 15 days after the emergence of the plumules above ground. Seedlings germinated in an ice box at 6°C. showed no infection;

173 seedlings which had germinated in a cool damp place north of the greenhouse at a temperature ranging about 15°C. showed 8.8 percent infection; and 8.1 percent of a similar number of plants germinated at about 26°C. on a greenhouse bench showed positive symptoms of infection.

Only a small percentage of the infected corn plants were found to be sporulating under natural conditions. However, an abundance of conidia were produced from mottled portions of diseased leaves by covering the plants at night with inverted glass jars. The plants most completely invaded by the mildew died before pushing out the third leaf, while others remained alive in a dwarfed condition thruout the season or apparently became free from the disease. Fig. 7 (left) shows two plants of popcorn (Japanese Hulless) grown in the field tests in 1927. The tall plant is normal while the stunted plant is infected with Sclerospora.

In Iowa, *Sclerospora graminicola* has been observed only twice occurring naturally on corn in the field; on dent corn seedlings at Boone in 1925, and on sweet corn (Golden Bantam) in the ear stage at Story City in 1927 (fig. 7).

A survey made in June, 1927, which included readings on 3,350 corn plants (dent, pop and sweet) located at 19 places in 16 of the north central counties of Iowa, revealed no positive symptoms of infection of Sclerospora.

Setaria viridis

Conidia of *Sclerospora graminicola* have been observed to develop on the first seedlings of *Setaria viridis* in the spring and to appear at various times thruout the growing season until checked by frost.

Downy mildew was discovered on a few plants of *Setaria viridis* on October 14, 1924. Conidia were washed from infected leaves at 6:30 a.m. for germination trials, but they failed to produce zoospores. At 9:50 a.m. the remaining conidiophores were removed from the leaves and a fresh crop of spores was allowed to develop when the leaves were placed in a moist chamber. Of these, 60 percent germinated. No other conidia were observed in the open at a later date that year. The spring of 1925 was drier than usual in Iowa. However, mildew was found on May 16 on the first seedlings of *Setaria viridis* to appear.

The first oospores collected in 1925 were found in a spike of *Setaria viridis* on July 8. However, no shredded heads were observed before July 20. A quantity of oospores gathered July 23 was used to expose *S. viridis* seedlings in 25 small pots. These were transplanted in the field on August 3 and showed heavy infection. The typical, rolled heads began to appear by the first of October and mature oospores were gathered on October 22. The unexposed check plants developed no infection. This test

TABLE XVIII. LONGEVITY OF OOSPORES OF SCLEROSPORA

No.	Oospores collected	Date seeds exposed	Interval	Hosts	Total plants exposed	Percent plants infected
1	1910	Apr., 1925	14½ yrs.	*Setaria viridis*	Many	0
2	" "	" "	" "	*Setaria italica*	"	0
3	Aug., 1924	Jan., 1926	17 mos.	Popcorn (*Zea mays*)	21	23
4	" "	" "	17 mos.	*Setaria viridis*	Many	20
5	" "	Feb., 1927	30 mos.	" "	"	10
6	Aug., 1925	" "	18 mos.	" "	"	19
7	" "	Mar., 1927	19 mos.	" "	1383	26
8	Sept., 1926	Feb., 1927	5 mos.	" "	Many	13
9	" "	Mar., 1927	6 mos.	" "	1027	52

indicates two crops of oospores may be produced during one growing season in Iowa.

LONGEVITY OF OOSPORES

It has already been demonstrated that *Sclerospora graminicola* in nature is carried from one year to the next by its oospores (table VIII) and that *Setaria viridis* was infected when planted in field soil taken up during the winter. As with other soil-borne diseases, the question naturally follows as to the length of time the oospores will retain their viability.

On March 3, 1925, two pots of soil infested oospores were planted with *Panicum miliaceum* (broom corn millet) and another pot with *Pennisetum typhoideum* (pearl millet). As no infection developed the plants were pulled and the pots were replanted with *Setaria viridis* on April 6. On April 18 several plants of the new planting showed disease symptoms. The pots had remained on the greenhouse bench continuously from the first planting.

This indicated that Sclerospora oospores might live in soil for at least 46 days.

Table XVIII shows infection experiments with oospores of different ages on *Setaria viridis, S. italica* and popcorn. The spores were placed on the seed in the soil pot cultures and conditions made favorable for infection. Oospores which were collected by the senior author at Madison, Wisconsin, in 1910 produced no infection. Other oospores which were collected in August, 1924, and were 30 months old at the time they were used, produced 10 percent infection. Whether oospores can retain their viability in the soil in the field for such lengths of time is not known.

Most of the diseased plants observed during the summer of 1925 appeared to be systemically infected. This seems to be quite typical of infection caused by oospores. It is probable that the fungus is carried over the winter by its oospores which lie on and in the ground. The spores do not germinate at once but may infect seedlings at any time during the growing season.

The extent of secondary infection in the field is uncertain.

Conidial infection has proved to be difficult to induce in the greenhouse. In flats where rows of heavily infected plants of Setaria have grown side by side with unexposed check rows, no spontaneous spread of the disease has ever been observed.

However, the function of the conidia must not be under estimated because systemic infection of *Setaria viridis* and *S. italica* has been produced by conidial exposure of plants growing in sterile soil. These infected plants have later produced oospores in the typical manner, both upon *S. viridis* and *S. italica.*

INFLUENCE OF CERTAIN CONDITIONS ON OOSPORE GERMINATION

The process involved in the germination of Sclerospora oospores has remained in obscurity thruout the researches of workers for many years. The simple experiment of placing oospores on seeds of *Setaria viridis* in sterile soil and later finding the seedlings completely invaded with the mycelium of the mildew is sufficient evidence to conclude that the oospores do germinate. However, the method by which this is accomplished is still uncertain.

Soil Reaction in Relation to Infection

In a preliminary study, a series of seeds of popcorn (Yellow Pearl) exposed to oospores was watered with various concentrations of sodium hydroxide and hydrochloric acid solutions. The purpose of the experiment was to ascertain whether infection was favored by an alkaline or acid condition. The trials were run in triplicate and about 100 cc. of solution were added to each pot. Table XIX shows that the solutions used did not greatly reduce infection.

Soaking in Water

A number of attempts were made to test the role of water in the germination of oospores. Thinking that the spores in water cultures might be germinating, but in an unfamiliar manner, the authors set about to find the influence of presoaking on infection.

Table XX shows little significant difference in the amount of

TABLE XIX. EFFECT OF DILUTE SODIUM HYDROXIDE AND HYDROCHLORIC ACID ON OOSPORE INFECTION OF *ZEA MAYS*

Solution (Percent)	Total plants exposed	Number plants infected	Percent plants infected
0.5 NaOH	30	22	73
0.1 "	29	22	76
0.05 "	32	18	56
0.01 "	28	17	61
1.0 HCl	33	13	40
0.5 "	30	21	70
0.1 "	31	18	58
0.05 "	32	21	66
Ck: Tap water	28	19	68

TABLE XX. THE AMOUNT OF INFECTION OBTAINED BY PRE-SOAKED OOSPORES

No.	Date of collection	Length of soaking	Total plants	Percent plants infected
1	1925	7 days	184	11.4
2	"	6 "	137	28.5
3	"	5 "	245	28.6
4	"	4 "	209	27.3
5	"	3 "	190	28.9
6	"	21 hrs.	142	28.2
7	"	13 hrs.	188	25.5
8	"	Ck. no soaking	92	29.3
9	1926	7 days	132	49.1
10	"	6 "	166	46.0
11	"	5 "	133	47.4
12	"	4 "	119	50.4
13	"	3 "	64	56.3
14	"	21 hrs.	201	56.7
15	"	13 hrs.	91	63.7
16	"	Ck. no soaking	110	41.8

infection obtained from pre-soaked and dry oospores. Three interpretations may be given to these results: (a) oospores may not germinate when soaking in water because of the lack of oxygen; (b) soil may possess some physical or chemical property which aids in the process; (c) the host plant may exert some influence upon germination of the oospores, the probability being greater because the fungus is an obligate parasite.

Soil Not Necessary as a Medium for Germination

Seeds of *Setaria viridis* were covered with oospores and planted in cotton. The cultures were kept moist by hanging wicks down into distilled water below. In a portion of the experiment, a nutrient salt solution was used for watering the germinating seeds. This was prepared by adding 8.4 cc. M/1, KH_2PO_4, 24.85 cc. M/1 $Ca(NO_3)_2$, 32.2 cc. M/1 $MgSO_4$, and 2 cc. M/1 $FeCl_3$ to 3432.5 cc. of distilled water. Similar cultures were prepared in which sterile soil was used instead of cotton.

Table XXI shows that plants in each trial were infected and that sporulation appeared on the plants 12 days after planting. Practically the same percentage of disease was found among plants grown on cotton as on the control plants grown in soil. The nutrient solutions did not appear to influence oospore infection altho the plants watered by it possessed a more luxuriant growth than those grown in distilled water.

TABLE XXI. INFLUENCE OF SUBSTRATE ON INFECTION BY OOSPORES

No.	Host	Watering solution	Substrate	Percent infection
1	*Setaria viridis*	Dist. water	Cotton	20
2	" "	" "	"	60
3	" "	Nutrient	"	60
4	" "	"	"	60
5	" "	"	Sterile soil	50
6	" "	"	" "	40
7	" "	Dist. water	" "	75
8	" "	" "	" "	50

A similar test was made on popcorn (Japanese Hulless) and infection was induced in the "cotton" cultures as well as in the soil.

This experiment indicates that soil is not necessary for oospore germination. However, it is possible that the physical conditions of moisture and oxygen present in soil *are* necessary for germination and that these were afforded by aid of the cotton.

Influence of the Host on Germination of Oospores

This is perhaps the most difficult and yet the most important problem concerned with oospore germination. *Sclerospora graminicola* is an obligate parasite. In other words, the mycelium has never been grown except in intimate contact with its host. On the other hand, the conidia are easily induced to produce zoospores in water. And, from the vast amount of work which has been done on the germination of other fungus spores, there is little reason to believe that the *presence* of the host is essential for the mere germination of the oospores.

The possibility that the carbon dioxide given off by the seedling in its respiratory processes was responsible for the spore stimulation, led to an experiment in which definite percentages of this gas were supplied to the atmosphere of oospores. The apparatus and procedure used were similar to those described by Platz, Durrell and Howe (13) in their work on carbon-dioxide stimulation of corn smut spores.

Oospores sprinkled on sterile 3 percent agar were placed in atmospheres of 5 and 15 percent carbon dioxide, held at 20° to 22°C. for 72 hours. When removed and examined microscopically, these cultures were found to be worthless because of the contaminating fungous and bacterial growth. No germination of the oospores was revealed.

Discussion Concerning Oospore Germination

The elimination of contaminating organisms from oospores is a problem which apparently must be solved before the actual germination of oospores can be studied. Altho formed within the tissues of infected host plants, the shredding of the leaves in the field and the processes involved in the threshing, all permit the mixing of the spores of other fungi, bacteria and encysted protozoans with the oospores. When oospores are scattered on the surface of sterile media, contaminating fungous and bacterial growths soon obscure them. In hanging drop cultures the oospores are soon surrounded by hosts of protozoa and fungous hyphae. When oospores are mixed with moist sterile soil, the protozoa and bacteria soon become so active that microscopic examination is quite useless. Any possible products of oospore germination might well be confused with the contaminating organisms present, and, conversely, proof of the nature of oospore-

germination-products would be difficult to establish as long as contaminating organisms were present.

Considerable use was made of chemicals, heat and mechanical appliances to do away with the contaminating organisms. One process included the use of a centrifuge to separate out the lighter vascular constituents of the host tissue. This was followed by treatment with ½ percent copper sulfate and 1 to 10,000 mercuric chloride solutions interspersed by thoro washing in sterile water. Altho this procedure eliminated most of the contaminants, it failed to kill all of them. It further indicated that some of the foreign organisms present were more resistant to the disinfectants used than were the oospores.

Altho the oospores have never been observed to germinate, yet the studies that have been made make it possible to know some things about the process.

It would seem that in the soil only a small percentage of the oospores germinate at any one time. In exposing corn seeds to infection, the best results have been obtained after placing half of a cubic centimeter of oospores directly on the germ of the seed and many more thru the top soil. Merely dusting oospores over the seeds has seldom given high percentage of infection. It is, therefore, probable that only small percentages of the oospores germinate at any one time and this may well account for the failure of workers to actually observe the germination of oospores under the microscope.

Furthermore, artificially oospore-infested soil may produce infection in two or more successive crops of *Setaria viridis* without the addition of fresh oospores with each planting of seed. It would seem likely that only a few oospores germinate at one time even when held under ideal conditions for seed germination and plant growth.

The period of dormancy of oospores does not seem to be affected by soaking in distilled water, tap water or soil water. Oospores soaked in water thru which carbon dioxide was bubbled at frequent intervals did not infect seedlings of *Setaria viridis* more readily than did unsoaked oospores. Whether the thick oogonial wall surrounding the spore permits the imbibition of water is unknown, yet the infectibility of dry oospores would indicate that imbibition is not one of the primary stimuli for germination.

Freezing, or even a short period of dormancy, it seems, is not a prerequisite for germination. Oospores matured in July have been used again within 10 days to produce infection on a new crop of seedlings of *Setaria viridis*. In other cases oospores have remained viable for two and one-half years.

From these observations, it seems probable that the unknown stimulus for germination is not alone the imbibition of water, freezing or a rest period. Other conditions such as dry heating,

soaking in dilute chemical solutions and incubating in soil have not seemed especially to embody the desired stimulus. Of course, oospore germination may be brought about as the result of a number of conditions working together, but the fact that some infection of host plants occurs under such a diversity of conditions indicates that the factor or factors favoring germination do not have a narrow range.

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